

CLINICAL STUDY PROTOCOL

A single-arm, observational study to explore and characterize wound healing after skin punch biopsies in healthy volunteers

Short Title:	Wound healing in healthy volunteers
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Version:	1
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Date:	13-Sep-2017
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CHDR number:	CHDR1736
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Toetsing Online number:	NL63280.056.17
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EudraCT number:	Not applicable
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Study Title

A single-arm, observational study to explore and characterize wound healing after skin punch biopsies in healthy volunteers

I acknowledge responsibility for this protocol in accordance with CHDR's current procedures.

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LIST OF ABBREVIATIONS

AE	Adverse Event
ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee; in Dutch, ABR = Algemene Beoordeling en Registratie
ANCOVA	Analysis of Covariance
BMI	Body Mass Index
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CHDR	Centre for Human Drug Research
CRF	Case Report Form
EC	Ethics Committee (also Medical Research Ethics Committee (MREC); in Dutch: Medisch Ethische Toetsing Commissie (METC).
EDTA	Ethylene diamine tetra-acetic acid
EOS	End Of Study
EU	European Union
GCP	Good Clinical Practice
HE	hematoxylin and eosin
ICH	International Conference on Harmonization
IL	Interleukin
LSCI	Laser Speckle Contrast Image
MedDRA	Medical Dictionary for Regulatory Activities
NSAID	non-steroid anti-inflammatory drugs
NRS	Numeric Rating Scale
OTC	over-the-counter
PD	Pharmacodynamics
POSAS	Patient and Observer Scar Assessment Scale
qRT-PCR	real-time reverse transcription polymerase chain reaction
RNA-seq	RNA sequencing
RYB	Red-Yellow-Black wound assessment scale
SAE	Serious Adverse Event
SD	Standard Deviation
SEM	Standard Error of the Mean
SOC	System Organ Class
SOP	Standard Operating Procedure
SST	Serum Separator Tube
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAP	Transdermal analysis patch
TEWL	Trans Epidermal Water Loss
TNF	Tumor Necrosis Factor
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen.

PROTOCOL SYNOPSIS

Title

A single-arm, observational study to explore and characterize wound healing after skin punch biopsies in healthy volunteers

Short Title

Wound healing in healthy volunteers

Principal investigator & Trial Site

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Background & Rationale

The skin plays a critical role in protection where it acts as a barrier from damage and pathogens between the external and internal environments (Dreifke et al., 2015). Wounds compromise its protective role by disrupting the function and the normal structure of the skin and the underlying soft tissue. As a response to injury wound healing occurs in order to rapidly restore the defect. This process involves activation of, among others, keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets and consists of multiple phases including hemostasis, inflammation, migration and cellular proliferation, and maturation and remodeling (Armstrong and Meyr, 2017). A simplified schematic of the course of wound healing is depicted in Figure 2. Due to the broad involvement of various cell types, extracellular matrix and many reactive molecules each phase in wound healing produces characteristic changes within the tissue. A deficiency in any part of the process can lead to delayed wound healing, abnormal scar formation or chronic wounds.

To study wound healing in healthy volunteers a challenge model with skin punch biopsies has been described in literature previously (Greaves et al., 2014, Greaves et al., 2015, Illigens and Gibbons, 2013, Ud-Din et al., 2012). However, the characterization of this model was not performed comprehensively since advanced analysis of biopsies were omitted. Furthermore, analyses performed in previous studies only partially described wound healing processes either by insufficient time points for characterization or scarce simultaneous evaluations of multiple wound healing modalities. In addition, novel non-invasive imaging methodologies including thermography, 3D photography, multispectral imaging, enable more comprehensive profiling of the wound morphology.

The overall aim of this study is to develop a standardized model to temporarily and locally induce a skin trauma to investigate wound healing and monitor wound closure. This clinical model will enable future application as proof-of-pharmacology and proof-of concept studies as well as drug profiling in early drug development programs. More specifically, the objective of the trial is to explore and characterize the induction of well-defined skin trauma and natural wound healing process over the course of the different phases using a battery of dermatological assessments after skin punch biopsies in healthy volunteers. Furthermore, safety and tolerability will be assessed.

Characterization and monitoring of wound healing effects following skin punch biopsies will be performed by means of biophysical, biochemical, imaging, clinical parameters and subject reported outcomes.

Objective(s)**Primary Objective**

- To characterize and monitor wound healing after a standardized, induced skin trauma

Secondary Objectives

- To evaluate safety and tolerability of biopsy-induced skin trauma

Design

This is an observational, single center, single arm study.

Treatments

Biopsy sites will be treated by secondary healing intention with a non-stick gauze dressing followed by no treatment after 48h.

Investigational drug

Not applicable

Comparative drug

No comparative drug will be used in this study.

Study periods

The total duration of the study will be approximately 14 weeks: 4 weeks for screening and 10 weeks of observation. Subjects will visit CHDR 14 times. For a detailed outline see Table 1.

Subjects / Groups

A total of 18 healthy volunteers are planned to be enrolled. The study will entail 1 cohort with a randomized repeated biopsy collection time. Three skin punch biopsies (3 mm) of the lower back will be taken from each volunteer on day 0, with a distance of approximately 3-5 cm in between. All biopsy lesions will be treated with a gauze dressing (Jelonet®, paraffine gauze + Tegaderm®) for 48h after which the gauze is removed. Hereafter, the biopsy lesions will remain untreated. One biopsy sample taken on day 0 will serve as a baseline measurement for the repeated samples regarding the histology, immunohistochemistry, and RNA sequencing (RNA-seq) or real-time reverse transcription polymerase chain reaction (qRT-PCR) assessments.

Repeated biopsies of the same location as on day 0 will be taken on day 7, 14 or 21 (biopsy lesion and day randomized), and day 28, 42 or 56 (biopsy lesion and day randomized) for all subjects. The observation biopsy (biopsy lesion randomized) will serve as primary biopsy and followed for all measurements. All repeated biopsy lesions will also be treated with a gauze dressing (Jelonet®, paraffine gauze + Tegaderm®) for 48h after which the gauze is removed and observation commences.

Cohort	Observation period (day 2 - day 70)	Number of subjects
1	3 biopsies taken on day 0 <ul style="list-style-type: none"> - 1 repeated biopsy taken on day 7, 14 or 21 (randomized) - 1 repeated biopsy taken on day 28, 42 or 56 (randomized) - 1 target (primary) biopsy for observation without re-excision 	18

Inclusion criteria

Eligible subjects must meet all of the following inclusion criteria at screening:

1. Healthy subjects, 18 to 30 years of age (inclusive). The health status is verified by absence of evidence of any clinical significant active or uncontrolled chronic disease following a detailed medical history, a complete physical examination including vital signs, blood sampling of hematology, chemistry, and virology, urinalysis, urine drug and cotinine testing, and alcohol breath testing. In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility or judged to be clinically irrelevant for healthy subjects.
2. Body mass index (BMI) between 18 and 30 kg/m², inclusive
3. Fitzpatrick Skin type I-II (Caucasian type).
4. Eligible lower back to perform biopsies (no excessive hair growth, no local skin disorder)
5. Willing to give written informed consent and willing and able to comply with the study protocol.

Exclusion criteria

Eligible subjects must meet *none* of the following exclusion criteria at screening:

1. History of pathological scar formation (keloid, hypertrophic scars)
2. Any form of body modification of the lower back hindering biopsy collection of unaltered skin (e.g. tattoos, piercings, implants)
3. Any disease associated with immune system impairment, including auto-immune diseases, HIV and transplantation patients.
4. Requirement of immunosuppressive or immunomodulatory medication, including glucocorticoids, non-steroid anti-inflammatory drugs (NSAIDs), and chemotherapeutic drugs within 30 days prior to enrollment or planned to use during the course of the study.
5. Have any current and/or recurrent pathologically, clinical significant relevant skin condition.
6. Use of topical medication (prescription or over-the-counter (OTC)) within 30 days of the start of the study in local treatment area.
7. Pregnant, a positive pregnancy test, intending to become pregnant, or breastfeeding.
8. Current smoker and/or regular user of other nicotine-containing products (e.g., patches).
9. Average consumption of more than 14 units of alcohol per week
10. Tanning due to sunbathing, excessive sun exposure or a tanning booth within 3 weeks of enrollment or planned to do so during the course of the study
11. Participation in an investigational drug or device study within 3 months prior to screening or more than 4 times a year.
12. Loss or donation of blood over 500 mL within three months prior to screening.
13. Any (medical) condition that would, in the opinion of the investigator, potentially compromise the safety or compliance of the subject or may preclude the subjects' successful completion of the clinical trial.

Concomitant medications

No prescription medication or OTC medications (excluding multivitamins) will be permitted within 21 days prior to the start of the study, or less than 5 half-lives (whichever is longer), and during the course of the study. Exceptions are paracetamol (up to 4 g/day) in case of local pain. Use of pain medication will be determined by the investigator individually. Other exceptions will only be made if the rationale is discussed and clearly documented.

Endpoints

- Biopsy biomarkers:
 - Histology with hematoxylin and eosin (HE) staining
 - Immunohistochemistry with wound healing related biomarkers (e.g. CD31, collagen I, collagen III, αSMA, fibronectin)
 - RNA-seq or qRT-PCR for wound healing related biomarkers (e.g. VEGFα, TGFβ1, TGFβ2, TGFβ3, PDGF, CTGF, TNF, IL-1B, IL-4, GM-CSF, IL-6, IL-10, MMP1, MMP3, OSM, LOX)
- Local skin biomarkers for wound healing related biomarkers (e.g. VEGF-A, TNFα, IL-8, TLSP, MMP-3, IL-4) by transdermal analysis patch (TAP)
- Clinical imaging (e.g. 2D and 3D photography, thermography, laser speckle contrast imaging (LSCI), trans epidermal water loss (TEWL), colorimetry)
- Clinical evaluation (erythema grading scale, Red-Yellow-Black (RYB) wound assessment scale, the Patient and Observer Scar Assessment Scale (POSAS))
- Skin microbiome (healthy and biopsy lesions)

Tolerability / safety endpoints

- Adverse events (AEs)
- Local tolerance (erythema grading scale, RYB wound assessment scale, POSAS, NRS pruritus and pain)

All of above mentioned pharmacodynamic and safety measurements will be performed multiple times during the study according to the Visit and assessment schedule (Table 1).

Sample Size Justification

A total cohort size of 18 healthy volunteers will be investigated. This is justified since the primary objective is to explore and monitor the wound healing model. No formal power calculation was performed given the exploratory character of the study.

Statistical methodology

Data listings and averages will be presented for pharmacodynamics and safety measures.

Given the exploratory character of the study, pharmacodynamic endpoints will be primarily analyzed using descriptive statistics. All pharmacodynamic endpoints will be summarized (mean and standard deviation of the mean, median, minimum and maximum values) by time, and will also be presented graphically as mean over time, with standard deviation as error bars. Both Nominal results, and log-transformed results and change from baseline results will be utilized in all data summaries. All categorical pharmacodynamic endpoints will be summarized by frequencies.

Table 1: Visit and schedule assessment

	SCR	Day 0	Day 2	Day 4	Day 7	Day 10	Day 14	Day17	Day 21	Day 24	Day 28	Day 42	Day 56	Day 70
Assessment \ Time	Up to -28 days													EOS
Informed consent	X													
Demography (age, race, gender)	X													
Inclusion and exclusion criteria	X													
Medical history	X													
Physical examination	X													
Height and weight	X													
BsHem, BsChem, BsVir, UrSamStk, UrPregnancy, UrDrugSc, UrCotinine, BreathAlc	X													
Vital signs (HR, BP)	X													
Temperature	X													
General symptoms		X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical imaging (2D and 3D photography, thermography, LSCI, TEWL, colorimetry)		X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical assessments (erythema grading scale, RYB wound assessment scale, POSAS)		X	X	X	X	X	X	X	X	X	X	X	X	X
Subject assessment (pruritus, pain, POSAS)		X	X	X	X	X	X	X	X	X	X	X	X	X
TAP and skin microbiome (healthy and lesions)		X	X	X	X	X	X	X	X	X	X	X	X	X
Skin punch biopsy ^A		X			X ^B		X ^B		X ^B		X ^B	X ^B	X ^B	
(S)AE / ConMed	X	X	X	X	X	X	X	X	X	X	X	X	X	X

SCR = Screening; EOS = End of study; BsHem = Hematology blood sample; BsChem = Chemistry blood sample; BsVir = Virology blood sample; UrSamStk = Urinalysis; UrPregnancy = Urine sample pregnancy test for woman of childbearing potential; UrDrugSc = Urine drug test; UrCotinine = Urine cotinine test; BreathAlc = Alcohol breath test; HR = Heart rate; BP = Blood pressure; LSCI = Laser Speckle Contrast Imaging; TEWL = Transepidermal Water Loss; RYB = Red-Yellow-Black wound assessment scale; TAP = Transdermal Analysis Patch; (S)AE = (Serious) adverse event; ConMed = Concomitant medication

^A Biopsies taken after measurements are performed

^B Randomized repeated biopsy sample collection time, maximum 5 per subject over three site during the trial

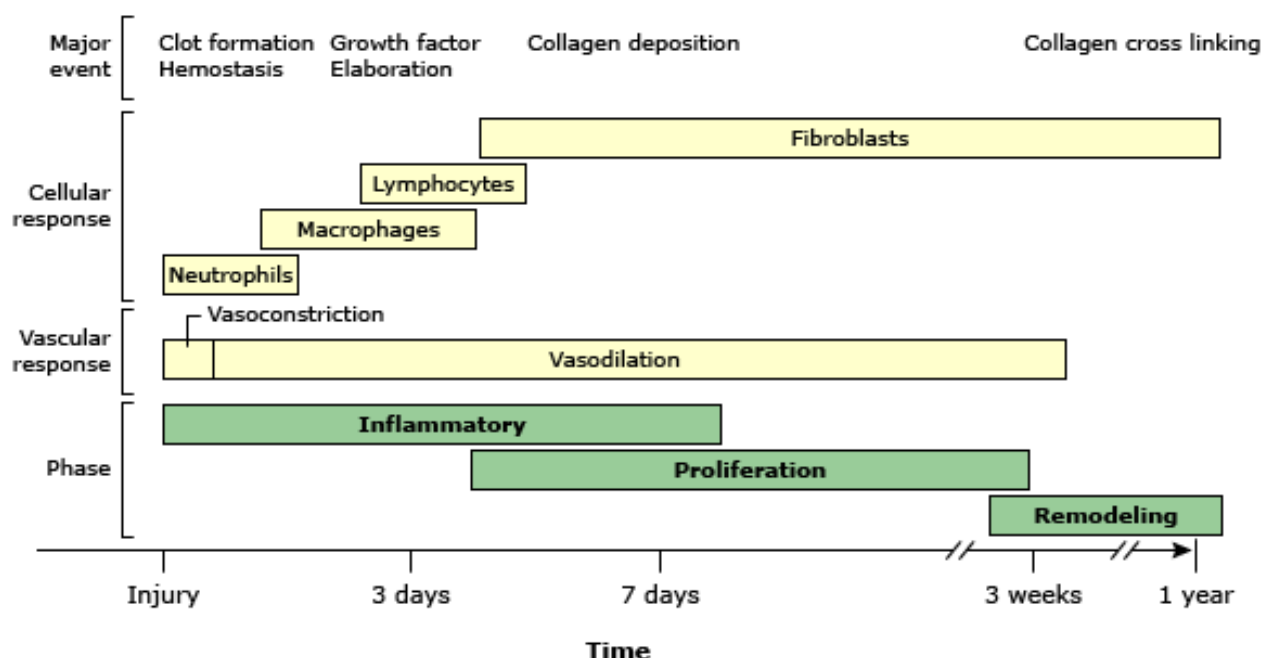
1 BACKGROUND AND RATIONALE

1.1 Context

The skin plays a critical role in protection where it acts as a barrier from damage and pathogens between the external and internal environments (Dreifke et al., 2015). Wounds compromise its protective role by disrupting the function and the normal structure of the skin and the underlying soft tissue. As a response to injury wound healing occurs in order to rapidly restore the defect. This process involves activation of keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets and consists of multiple phases including hemostasis, inflammation, migration and cellular proliferation, and maturation and remodeling (Armstrong and Meyr, 2017). A simplified schematic of the course of wound healing is depicted in Figure 2. Hemostasis occurs immediately after dermal injury. The inflammation phase is characterized by cellular recruitment and increased vascular permeability. The epithelization phase is achieved by proliferation of basal cells and migration of epithelial cells. The last phase is known as the maturation and remodeling phase where collagen cross-linking and remodeling, wound contraction, and repigmentation takes place. Due to the broad involvement of various cell types, extracellular matrix and many reactive molecules each phase in wound healing produces characteristic changes within the tissue. A deficiency in any part of the process can lead to delayed wound healing, abnormal scar formation or chronic wounds.

To study wound healing in healthy volunteers a challenge model with skin punch biopsies has been described in literature previously (Greaves et al., 2014, Greaves et al., 2015, Illigens and Gibbons, 2013, Ud-Din et al., 2012). However, the characterization of this model was not performed comprehensively since advanced analysis of biopsies were omitted. Furthermore, analyses performed in previous studies only partially described wound healing processes either by insufficient time points for characterization or scarce simultaneous evaluations of multiple wound healing modalities.

Figure 2: Wound healing time line



The overall aim of this study is to develop a standardized model to temporarily and locally induce a skin trauma to investigate wound healing and monitor wound closure. This clinical model will enable future application as proof-of-pharmacology and proof-of concept studies as well as drug profiling in early drug development programs. More specifically, the objective of the trial is to explore and characterize the induction of well-defined skin trauma and natural wound healing process over the

course of the different phases using a battery of dermatological assessments after skin punch biopsies in healthy volunteers. Furthermore, safety and tolerability will be assessed.

Characterization and monitoring of wound healing effects following skin punch biopsies will be performed by means of biophysical, biochemical, imaging, clinical parameters and subject reported outcomes.

1.2 Non-clinical information

No drug or treatment with active ingredients is to be used in this study.

1.3 Clinical information

No drug or treatment with active ingredients is to be used in this study.

1.4 Study rationale

1.4.1 Benefit and risk assessment

Small skin punch biopsies are frequently performed in both clinical care and clinical research. Skin punch biopsies of 3 mm and 4 mm are considered minimally invasive since the wound surface is limited and wound care does usually not involve sutures. The induced wounds heal quickly and do not cause tremendous scarring. Subjects with a high risk for hypertrophic scarring will be excluded.

Therefore, the risks associated with study participation are considered minimal.

1.4.2 Study population

For this trial 18 healthy Caucasian (Fitzpatrick Skin type I-II) volunteers between the age of 18 and 30 will be included.

1.4.3 Study design

This is an observational, single center, single arm study to monitor and characterize wound healing after skin punch biopsies in healthy volunteers. An extensive battery of tests of various dermatological assessments will advance the understanding of normal wound healing.

1.4.4 Comparative drug(s) and/or placebo

Not applicable.

1.4.5 Duration of observation

Based on previous wound healing studies an observation duration of 70 days is chosen (Greaves et al., 2014, Greaves et al., 2015, Illigens and Gibbons, 2013, Ud-Din et al., 2015, Ud-Din et al., 2012). This is appropriate since study objectives such as characterizing and monitoring wound healing can be assessed adequately.

1.4.6 Primary endpoint

Pharmacodynamic endpoints

- Biopsy biomarkers:

- Histology with hematoxylin and eosin (HE) staining
- Immunohistochemistry with wound healing related biomarkers (e.g. CD31, collagen I, collagen III, αSMA, fibronectin)
- RNA sequencing (RNA-seq) or qRT-PCR for wound healing related biomarkers (e.g. VEGFα, TGFβ1, TGFβ2, TGFβ3, PDGF, CTGF, TNF, IL-1B, IL-4, GM-CSF, IL-6, IL-10, MMP1, MMP3, OSM, LOX)
- Local skin biomarkers for wound healing related biomarkers (e.g. VEGF-A, TNFα, IL-8, TLSP, MMP-3, IL-4) by transdermal analysis patch (TAP)
- Clinical imaging (e.g. 2D and 3D photography, thermography, laser speckle contrast imaging (LSCI), trans epidermal water loss (TEWL), colorimetry)
- Clinical evaluation (erythema grading scale, Red-Yellow-Black (RYB) wound assessment scale, the Patient and Observer Scar Assessment Scale (POSAS))
- Skin microbiome (healthy and biopsy lesions)

Tolerability / safety endpoints

- Adverse events (AEs)
- Local tolerance (erythema grading scale, RYB wound assessment scale, NRS pruritus and pain, POSAS)

All of above mentioned pharmacodynamic and safety measurements will be performed multiple times during the study according to the Visit and Assessment Schedule (Table 1).

1.4.7 Statistical hypotheses and sample size

A total cohort size of 18 healthy volunteers will be investigated. This is justified since the primary objective is to explore and monitor the wound healing model. No formal power calculation was performed given the exploratory character of the study.

2 STUDY OBJECTIVES

2.1 Primary objective

- To characterize and monitor wound healing after a standardized, induced skin trauma

2.2 Secondary objectives

- To evaluate safety and tolerability of biopsy-induced skin trauma

3 STUDY DESIGN

3.1 Overall study design and plan

This is an observational, single center, single arm study to monitor wound healing, explore the effects and evaluate safety/tolerability following a standardized, biopsy-induced skin trauma in healthy volunteers. A total of three biopsies will be taken: one will be used as primary (observational) lesion and two will be randomized to be re-excised for histological examination and exploration of biomarkers.

The total duration of the study for each subject will be up to 98 days divided as follows:

- Screening: Up to 28 days prior to first study day;
- In clinic visits: Days 0, 2, 4, 7, 10, 14, 17, 21, 24, 28, 42, 56, 70

3.1.1 Screening

Within 4 weeks prior to study baseline visit (day 0), volunteers will undergo a medical screening. Screening will be performed in a fasting state (≥ 4 hours), and consists of medical history, physical examination, height, weight, vital signs, temperature, blood sampling (hematology, biochemistry, virology), urinalysis, urine pregnancy testing (women only), urine drug and cotinine testing, and alcohol breath testing.

In addition, skin types will be assessed according to the Fitzpatrick classification.

Fitzpatrick skin type classification:

I: Pale white; blond or red hair; blue eyes; freckles — always burns, never tans

II: White; fair; blond or red hair; blue, green, or hazel eyes — usually burns, tans minimally

III: Cream white; fair with any hair or eye color; quite common — sometimes mild burn, tans uniformly

IV: Moderate brown; typical Mediterranean skin tone — sometimes mild burns, always tans well

V: Dark brown; Middle Eastern skin types — Very rarely burns, tans very easily

VI: Deeply pigmented dark brown to darkest brown— never burns, never tans

3.1.2 Observation period

The total observation period of the trial will be 10 weeks (70 days). Subjects will visit the clinical unit as specified in section 3.1.

Assessments will be performed on day 0, pre-dose on day 2, 4, 7, 10, 14, 17, 21, 24, 28, 42, 56 and 70. Safety and tolerability will be assessed throughout the study via questionnaires, clinical grading scales, and adverse event reporting.

All study procedure performed pre-dose and during the observation period are outlined in Table 1. Visit variances are allowed as described in Table 2.

4 STUDY POPULATION

4.1 Subject population

A total of 18 healthy Caucasian subjects will be enrolled into the study following satisfactory completion of a screening visit where eligibility for the study will be checked.

Subjects will be recruited via media advertisement or from the subjects' database of the Centre for Human Drug Research, Leiden, The Netherlands.

4.2 Inclusion criteria

Eligible subjects must meet all of the following inclusion criteria at screening:

1. Healthy subjects, 18 to 30 years of age (inclusive). The health status is verified by absence of evidence of any clinical significant active or uncontrolled chronic disease following a detailed medical history, a complete physical examination including vital signs, blood sampling of hematology, chemistry, and virology, urinalysis, urine drug and cotinine testing, and alcohol breath testing. In the case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility or judged to be clinically irrelevant for healthy subjects.
2. Body mass index (BMI) between 18 and 30 kg/m², inclusive
3. Fitzpatrick Skin type I-II (Caucasian type).
4. Eligible lower back to perform biopsies (no excessive hair growth, no local skin disorder)
5. Willing to give written informed consent and willing and able to comply with the study protocol.

4.3 Exclusion criteria

Eligible subjects must meet *none* of the following exclusion criteria at screening:

1. History of pathological scar formation (keloid, hypertrophic scars)
2. Any form of body modification of the lower back hindering biopsy collection of unaltered skin (e.g. tattoos, piercings, implants)
3. Any disease associated with immune system impairment, including auto-immune diseases, HIV and transplantation patients.
4. Requirement of immunosuppressive or immunomodulatory medication, including glucocorticoids, non-steroid anti-inflammatory drugs (NSAIDs), and chemotherapeutic drugs within 30 days prior to enrollment or planned to use during the course of the study.
5. Have any current and / or recurrent pathologically, clinical significant relevant skin condition.
6. Use of topical medication (prescription or over-the-counter (OTC)) within 30 days of the start of the study in local treatment area.
7. Pregnant, a positive pregnancy test, intending to become pregnant, or breastfeeding.
8. Current smoker and/or regular user of other nicotine-containing products (e.g., patches).
9. Average consumption of more than 14 units of alcohol per week
10. Tanning due to sunbathing, excessive sun exposure or a tanning booth within 3 weeks of enrollment or planned to do so during the course of the study
11. Participation in an investigational drug or device study within 3 months prior to screening or more than 4 times a year.
12. Loss or donation of blood over 500 mL within three months prior to screening.
13. Any (medical) condition that would, in the opinion of the investigator, potentially compromise the safety or compliance of the subject or may preclude the subjects' successful completion of the clinical trial.

4.4 Concomitant medications

All medications (prescription and over-the-counter (OTC)) taken within 30 days of study screening will be recorded.

No prescription medication or OTC medications (excluding multivitamins) will be permitted within 21 days prior to the first study day, or less than 5 half-lives (whichever is longer), and during the course of the study. Exceptions are paracetamol (up to 4 g/day) in case of local pain. Use of pain medication will be determined by the investigator individually. Other exceptions will only be made if the rationale is discussed and clearly documented.

4.5 Lifestyle restrictions

In the interest of their safety and to facilitate assessment, the subjects participating in this study will be requested to agree to the following restrictions during the study:

- Subjects should avoid prolonged exposure of involved skin to sunlight from three weeks prior to the study until EOS visit
- Bathing and washing of involved skin is not allowed 6 hours prior to each study visit.
- Alcohol will not be allowed from at least 24 hours before screening and before each scheduled visit. At other times throughout the study, subjects should not consume more than 2 units of alcohol daily on average (one unit is 10 grams of alcohol).
- Subjects will abstain from the use of tobacco-or nicotine-containing products (including e-cigarettes and patches) from the screening visit until the EOS visit.
- Female subjects must use effective contraception for the duration of the study.

4.6 Study discontinuation and withdrawal

4.6.1 Study drug interruption or discontinuation

The investigator must temporarily interrupt or permanently discontinue the study if continued participation is believed to be contrary to the best interests of the subject. The interruption or premature discontinuation might be triggered by an Adverse Event (AE), a diagnostic or therapeutic procedure, an abnormal assessment, or for administrative reasons in particular withdrawal of the subject's consent. The reason for interruption or premature discontinuation must be documented.

4.6.2 Subject withdrawal

Subjects have the right to withdraw from the study at any time for any reason. Should a subject decide to withdraw from the study, all efforts should be made to complete and report the observations, particularly the follow-up examinations, as thoroughly as possible.

4.6.3 Replacement policy

Subjects withdrawing for reasons other than adverse events or any other tolerability issues might be replaced.

5 INVESTIGATIONAL MEDICINAL PRODUCT

5.1 Investigational drug

Not applicable.

5.2 Study drug up- and down-titration

Not applicable.

5.3 Study drug packaging and labelling

Not applicable.

5.4 Example label in Dutch

Not applicable.

5.5 Drug accountability

Not applicable.

5.6 Treatment assignment and blinding

5.6.1 Randomization and treatment assignment

No treatment will be administered. Subjects in this study will be numbered sequentially from 1 to 18 in order of inclusion. Replacements will be numbered +100 (e.g. replacement for subject 1 will receive number 101).

The time of repeated biopsies will be randomized. The randomization code will be generated using SAS v9 by a study-independent, CHDR statistician.

5.6.2 Blinding

Not applicable.

6 STUDY ENDPOINTS

6.1 Endpoints

Wound healing will be assessed at the time points indicated in Table 1.

- Biopsy biomarkers:
 - Histology with hematoxylin and eosin (HE) staining
 - Immunohistochemistry with wound healing related biomarkers (e.g. CD31, collagen I, collagen III, αSMA, fibronectin)
 - RNA-seq or qRT-PCR for wound healing related biomarkers (e.g. VEGFα, TGFβ1, TGFβ2, TGFβ3, PDGF, CTGF, TNF, IL-1B, IL-6, IL-10, MMP1, MMP3, OSM, LOX)
- Local skin biomarkers for wound healing related biomarkers (e.g. VEGF-A, TNFα, IL-8, TLSP, MMP-3, IL-4) by transdermal analysis patch (TAP)
- Clinical imaging (e.g. 2D and 3D photography, thermography, laser speckle contrast imaging (LSCI), trans epidermal water loss (TEWL), colorimetry)
- Clinical evaluation (erythema grading scale, Red-Yellow-Black (RYB) wound assessment scale, the Patient and Observer Scar Assessment Scale (POSAS))
- Skin microbiome (healthy and biopsy lesion)

6.2 Safety and tolerability endpoints

Adverse events (AE) will be collected throughout the study, at every study visit.

- (serious) adverse events ((S)AEs).
- Concomitant medication
- Local tolerability (erythema grading scale, RYB wound assessment scale, POSAS, NRS pruritus and pain)

7 STUDY ASSESSMENTS

See the Visit and assessment schedule (Table 1) for the time points of the assessments.

7.1 Assessments

7.1.1 Biopsy sample collection

Initial biopsies

Three skin punch biopsies (3 mm) of the lower back will be taken from each volunteer on day 0, with a distance of approximately 3-5 cm in between. All biopsy lesions will be treated with a gauze dressing (Jelonet®, paraffine gauze + Tegaderm®) for 48h after which the gauze is removed. Hereafter, the biopsy lesions will remain untreated. One biopsy sample taken on day 0 will serve as a baseline measurement for the repeated samples regarding the histology, immunohistochemistry, and RNA sequencing (RNA-seq) or real-time reverse transcription polymerase chain reaction (qRT-PCR) assessments.

Repeated biopsies

Repeated biopsies of the same location as on day 0 will be taken on day 7, 14 or 21 (biopsy lesion and day randomized), and day 28, 42 or 56 (biopsy lesion and day randomized) for all subjects. The observation biopsy (biopsy lesion randomized) will serve as primary biopsy and followed for all measurements. All repeated biopsy lesions will also be treated with a gauze dressing (Jelonet®, paraffine gauze + Tegaderm®) for 48h after which the gauze is removed and observation commences.

The biopsy procedure will be performed according to the standard operating procedure for skin punch biopsies with local anaesthetics (CGESP BIO).

The biopsies will be placed in sealed tubes and frozen in liquid nitrogen immediately at a temperature of less than -160°C. The biopsy samples will be stored at CHDR in a -80°C freezer until shipment. The biopsy samples will be analyzed at the Immunology/pathology Laboratory at Erasmus MC, Rotterdam, The Netherlands for histology and biomarkers.

7.1.2 Histology

Collected biopsy samples will be analyzed at Erasmus MC, Rotterdam, The Netherlands. A histopathological score of the biopsies will be obtained using HE stained tissue multiple times during the study (see Table 1). Skin response will be assessed using the histological parameters characteristics of wound healing (amount of granulation tissue, inflammatory infiltrate, collagen fiber orientation, pattern of collagen, amount of early collagen, amount of mature collagen, migration of keratinocytes, bridging of cells, keratinization, new vessel formation).

The histopathological score for each of the patterns will be graded on a scale from 0 to 3.

7.1.3 Immunohistochemistry

Serial sections of biopsy samples will be stained with antibodies to identify wound healing related biomarkers (e.g. CD31, collagen I, collagen III, αSMA, fibronectin), and assessed by immunohistochemistry at Erasmus MC, Rotterdam, The Netherlands.

Biomarker analyses are subject to change and additional analyses may be performed in already collected materials.

7.1.4 Local biomarker sequencing

RNA-seq or qRT-PCR will be performed on collected biopsy samples for wound healing related biomarkers (e.g. VEGF α , TGF β 1, TGF β 2, TGF β 3, PDGF, CTGF, TNF, IL-1B, IL-6, IL-10, MMP1, MMP3, OSM, LOX) at Erasmus MC, Rotterdam, The Netherlands.

Biomarker analyses are subject to change and additional analyses may be performed in already collected materials.

7.1.5 Transdermal analysis patch (TAP)

Skin biomarkers will be measured explorative by TAP (FibroTx, Estonia) as indicated in Table 1. TAP consists of a multiplex capture-antibody micro-array that is supported by a dermal adhesive bandage for fixture to skin. When TAP is applied to skin and left on for 20 minutes, the antibodies printed on the micro-array capture biomarkers from skin through immune-recognition. Biomarkers (e.g. VEGF-A, TNF α , IL-4, IL-8, TSLP, MMP-3) captured from skin by TAP are qualitatively and quantitatively analyzed by spot-ELISA by a specific TAP analyzer. Each TAP kit will be labelled and stored at 4 °C overnight and after that frozen at -20°C until shipment.

Biomarker analyses are subject to change and additional analyses may be performed.

7.1.6 Skin microbiome

Sample collection

Collection of skin culture samples is a non-invasive procedure where a sterile polyester flock tip (Puritan Sterile Polyester Tipped Applicators REF 25-3206-H 20MM) per site is passed along the surface of the 3 different areas. The target areas are i) regions surrounding one of the biopsy lesions on the lower back, ii) a control site of healthy, unaffected skin in proximity of a biopsy lesion and iii) a control area on the lower back with a minimum distance of 10cm from a biopsy site. The skin swab will be placed in a 2 ml lysis tube (REF ZY-R1103, Zymo Research) containing DNA/RNA shield to stabilize and preserve the DNA. The tubes will be stored in the freezer at -80°C, to be shipped to BaseClear at the end of the study. The microbiology samples will be analyzed at BaseClear Laboratories, The Netherlands.

DNA extraction

The DNA extraction will be performed using adapted DNA extraction method based on the Zymo Research fecal DNA extraction methodology. In short, the swabs in the 2 ml lysis tubes undergo a mechanical shearing procedure that lyses cells from micro-organisms captured by the swab. DNA will be eluted in a volume of 50 μ l.

Microbiome analysis

After DNA extraction, the variable regions 3 and 4 of the 16S rRNA gene are amplified giving an amplicon of around 450 base pairs. This amplicon is analyzed by capillary systems using standard protocols, to confirm successful amplification of a PCR fragment of the expected size. PCR products are cleaned up by Ampure XP beads (Beckman Coulter) to remove primer-dimers and small a-specific PCR products and the purified PCR products are quantified using the Quant-iT PicoGreen dsDNA kit (Life Technologies). Subsequently, the PCR products are diluted and an equal mass for each sample is used as template in a 2nd PCR where sample specific barcodes (Index primers (Nextera). XT Index kit) will be appended to the PCR products using a 2nd PCR with a limited number of cycles. Following an PCR purification step as above, the PCR products are equimolarly

pooled and sequenced on the Illumina MiSeq platform using the MiSeq v3 sequencing kit generating paired end 300 nt sequence reads. De-multiplexed FASTQ files are generated as output. Paired end reads are assembly into 'pseudoreads' followed by removal of chimeric sequences and taxonomic classification. In addition, sequence reads are clustered together based on sequence similarity.

7.1.7 Clinical photography

On every visit day a standardized set of photographs will be taken of the treatment sites under standardized conditions (environment, light, distance) with FotoFinder ATBM and digital camera. Photographs will include a label with subject initials, subject study number, and study day/occasion number. All the photographs will be taken with QPcard 201 for later colour correction with image J software.

Pictures will also be taken and analyzed by other digital photosystems including a 3D stereo camera system (LifeViz QuantifiCare, Valbonne, France) and an Antera 3D camera system (Miravex, Dublin, Ireland).

7.1.8 Skin temperature by thermography

Skin temperature will be measured using a thermal imaging camera (FLIR X6540sc, FLIR Systems Inc., Breda, The Netherlands) according to the Table of Assessments (Table 1).

7.1.9 Perfusion by Laser spectrum contrast imaging (LSCI)

Cutaneous microcirculation will be assessed using the laser speckle imager (LSCI; PeriCam PSI System, Perimed Järfälla, Sweden), and will be done according to the SOP of CHDR at multiple time points during the study (see Table 1). Measurements have to be performed in a temperature controlled room with a temperature around 22°C. The subject has to get accommodated to the room temperature for a minimum of 15 minutes prior to testing. After this, the speckle assessments can commence. Briefly, the subject will be resting for at least ten minutes before any measurements take place. A suitable area of the lower back will be identified. This area will be illuminated by the laser and the response signal will be captured. Analysis of the data will be according to the pertaining SOP.

7.1.10 Trans epidermal water loss (TEWL)

To assess the barrier status of the skin TEWL will be measured at multiple time points during the study (see Table 1). The water loss will be measured non-invasively using an Aquaflux AF200 system (Biox, London, UK) according to the standard operating procedure of CHDR. A circular area of 7mm diameter of skin will be enclosed by the measuring probe. The flux of water that enters the chamber will be measured until a steady-state flux is reached. Minimal measurement time is 90 seconds and maximum measurement time is 200 seconds. All measurements will be performed under standard environmental conditions (temperature 22°C±2°C; relative humidity <60%), and subjects will be acclimatized under relaxed conditions for at least 15 minutes prior to testing.

7.1.11 Skin colour by colorimetry

Colorimetric assessments will be done and erythema grade will be calculated (a* of CIELAB, average of 3 measurements) by DSM II ColorMeter (Cortex Technology, Denmark).

7.1.12 Clinical grading scales

Skin colour will be assessed using an erythema grading scale provided by the supplier of the Finn Chambers. Wound healing will be assessed using Red-Yellow-Black (RYB) wound assessment scale and the Patient and Observer Scar Assessment Scale (POSAS).

The RYB wound assessment scale was introduced in the 1980s in order to simplify the assessments of wounds and to guide treatment. Classification of wounds with RYB model combined with the humidity of the wound is an approach used to categorize wounds and apply treatment (see below).

Wound colour Wound humidity	Red	Yellow	Black
Wet	Protect Absorb fluid	Cleanse Absorb fluid	Remove necrosis Absorb fluid
Humid	Protect Regulate fluid	Cleanse Regulate fluid	Remove necrosis Regulate fluid
Dry	Protect Apply fluid	Cleanse Apply fluid	Remove necrosis Apply fluid

The POSAS is a questionnaire developed in 2004 to measure scar quality in the opinion of the patient and the observer. The POSAS observer scale is provided in English in Appendix A and in Dutch in Appendix B. The location of the scar can be designated in a representative drawing of the human body.

The observer scale of the POSAS consists of six items (vascularity, pigmentation, thickness, relief, pliability and surface area). All items are scored on a scale ranging from 1 ('like normal skin') to 10 ('worst scar imaginable'). The sum of the six items results in a total score of the POSAS observer scale. Categories boxes are added for each item:

- Vascularity category: pale, pink, red, purple, mix
- Pigmentation category: hypo, hyper, mix
- Thickness category: thicker, thinner
- Relief category: more, less, mix
- Surface area category: expansion, contraction, mix

Furthermore, an overall opinion is scored on a scale ranging from 1 to 10.

All parameters should preferably be compared to normal skin on a comparable anatomic location.

7.1.13 Subject reported outcomes

Subjects are asked to report the pruritus and pain by a numeric rating scale of all biopsy sites. The pruritus and pain NRS are single-question assessment tools that will be used to assess the subject's worst itch and pain in the previous time interval. Subjects will fill in the e-diary daily and be asked the following question; "on a scale of 0 - 100, with 0 being no itch, and 100 being the worst itch imaginable, how would you rate your average degree of itch of all biopsy sites combined experienced during the previous time interval?" and "on a scale of 0 - 100, with 0 being no pain, and 100 being the worst pain imaginable, how would you rate your average degree of pain of all biopsy

sites combined experienced during the previous time interval?" Depending on visit day the time interval will be 2,3, 7, 14 days. Subjects will be instructed on reporting at the day 0 visit. Subjects will complete the pruritus and pain on every study visit through the last study visit (EOS).

7.2 Safety and tolerability assessments

The definitions, reporting and follow-up of AEs, SAEs and potential pregnancies are described in section 8.

7.2.1 Vital signs

Evaluation of systolic and diastolic blood pressure, pulse rate and temperature will be performed at screening as stated in Table 1. Pulse and blood pressure will be taken after 5 minutes in the supine position. Automated oscillometric blood pressures will be measured using a Dash 3000, Dash 4000, Dynamap 400 or Dynamap ProCare 400.

7.2.2 Weight and height

Weight (kg) and height (cm) will be recorded and body mass index (BMI) will be calculated at screening.

7.2.3 Physical examination

Physical examination (i.e., inspection, percussion, palpation and auscultation) is performed during screening. Clinically relevant findings that are present prior to study drug initiation must be recorded with the subject's Medical History.

7.2.4 Laboratory assessments

Following clinical laboratory tests will be performed at screening:

Lab	Tests	Collection & Analysis
Haematology	Haemoglobin [including Mean Corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC)], haematocrit, red cell count (RBC), total white cell count (WBC) and Platelet count. Differential blood count, including: basophils, eosinophils, neutrophils, lymphocytes, and monocytes.	4 mL of venous blood in a BD Vacutainer® K2EDTA tube. Samples will be analysed by the Clinical Chemistry Laboratory (AKCL) of Leiden University Medical Center.
Chemistry and electrolytes	Sodium, potassium, calcium, inorganic phosphate, total protein, albumin, glucose ¹ , triglycerides, blood urea nitrogen (BUN), creatinine, uric acid, total bilirubin ² , alkaline phosphatase, AST, ALT gamma-GT, LDH, and CDT.	8.5 mL of venous blood in a BD Vacutainer® SST Gel and Clot Activator tube. Samples will be analysed by the AKCL of Leiden University Medical Center.
Serology	HIV1 and HIV2 antigen and antibodies, Hepatitis B surface antigen, Hepatitis B antibodies and Hepatitis C antibodies	5 mL of venous blood in a BD Vacutainer® SST Gel and Clot Activator tube. Samples will be analysed by the Microbiology

		Laboratory (CKML) of the Leiden University Medical Center.
Urinalysis	Leucocytes, blood, nitrite, protein, urobilinogen, bilirubin, pH, specific gravity, ketones, glucose. If there is a clinically significant positive result, urine will be sent to the AKCL for microscopy and/or culture.	A midstream, clean-catch urine specimen will be analysed by dipstick (Multistix® 10 SG, Siemens Healthcare Diagnostics, Frimley, UK).
Pregnancy ³	hCG. If there is a clinically significant, positive result, urine will be sent to the AKCL for confirmation.	A urine specimen will be analysed at CHDR by test kit (InstAlert, Innovacon, San Diego, USA).
Alcohol	Alcohol Breath Test	The hand-held Alco-Sensor IV meter (Honac, Apeldoorn, the Netherlands) will be used to measure the breath ethanol concentrations.
Urine drug screen	Cocaine, amphetamines, opiates (morphine), benzodiazepines and cannabinoids.	A urine specimen will be analysed at CHDR by test kit (InstAlert, Innovacon, San Diego, USA).
¹ After 4-hours fasting. ² Conjugated bilirubin will be reported only when total bilirubin is outside the reference range. ³ Pregnancy test for women of childbearing potential will be performed at screening and if pregnancy is suspected during the study.		

7.2.5 Shipping Procedures

CHDR will arrange shipment of the biopsy samples. The samples must be packed securely together with completed shipment forms in polystyrene insulated shipping containers together with enough dry ice to last for 48 hours. Samples must be shipped to the Immunology Laboratory at Erasmus MC, Rotterdam.

7.2.6 Concomitant medications

Concomitant medications initiated, stopped, up-titrated or down-titrated for an AE will be recorded.

7.2.7 Local tolerability

The erythema grading scale, RYB wound assessment scale, POSAS, and NRS pruritus and pain will also be used to assess local tolerability.

7.3 Sequence of assessments and time windows

On study day 0 pharmacodynamic assessments will be performed prior to skin punch biopsies.

The deviations of actual time points from the expected time points will be within ten percent, calculated from the zero point (time of skin punch biopsy) or the last relevant activity. Deviations of more than 10% will be explained in a note. Pre-dose assessments are given in indicative expected times.

Visit variances are given in Table 2.

Table 2: Visit variances

Protocol procedure	Approved time window
Day 0	Per protocol
Day 2	Per protocol
Day 4	Per protocol
Day 7, Day 10	May be done plus or minus one (1) calendar days from scheduled
Day 14, Day 17	May be done plus or minus two (2) calendar days from scheduled
Day 21, Day 24	May be done plus or minus two (3) calendar days from scheduled
Day 28	May be done plus or minus two (4) calendar days from scheduled
Day 42	May be done plus or minus three (5) calendar days from scheduled
Day 56, Day 70	May be done plus or minus three (7) calendar days from scheduled

8 SAFETY REPORTING

8.1 Definitions of adverse events

An Adverse Event (AE) is any untoward medical occurrence in a subject who is participating in a clinical study performed. The AE does not necessarily have to follow the administration of a study drug, or to have a causal relationship with the study drug. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory or vital sign finding), symptom, or disease temporally associated with the study participation, whether or not it is related to the study drug.

8.1.1 Intensity of adverse events

The intensity of clinical AEs is graded three-point scale as defined below:

- Mild: discomfort noticed but no disruption of normal daily activity;
- Moderate: discomfort sufficient to reduce or affect normal daily activity;
- Severe: inability to work or perform daily activity.

8.1.2 Relationship to the intervention

For each AE the relationship to drug as judged by the investigator:

- Probable;
- Possible;
- Unlikely;
- Unrelated.

8.1.3 Chronicity of adverse events

The chronicity of the AE will be classified by the investigator on a three-item scale as defined below:

- Single occasion: single event with limited duration;
- Intermittent: several episodes of an event, each of limited duration;
- Persistent: event which remained indefinitely.

8.1.4 Action

Eventual actions taken will be recorded.

8.1.5 Serious adverse events

A Serious Adverse Event (SAE) is defined by the International Conference on Harmonization (ICH) guidelines as any AE fulfilling at least one of the following criteria:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or

- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a SAE.

8.1.6 Suspected unexpected serious adverse reactions

A SUSAR (Suspected Unexpected Serious Adverse Reaction) is a SAE that is unexpected, (nature or severity of which is not consistent with the applicable product information (e.g., investigator's brochure for an unauthorized investigational product or summary of product characteristics for an authorized product)) and suspected (a reasonable possibility of causal relationship with investigational drug, regardless of the administered dose).

8.1.7 Reporting of serious adverse events

SAEs and SUSAR's will be reported according to the following procedure, according to CHDR SOPs CGEAE and CGEEMERG.

The investigator will report the SAEs through the web portal ToetsingOnline (see <https://toetsingonline.nl/>) to the accredited EC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the SAE.

SUSARS must be reported to the EC that approved the study, the CA and the Dutch Medicines Evaluation Board (College ter Beoordeling van Geneesmiddelen).

The investigator will report expedited the following SUSARs through the web portal ToetsingOnline to the EC:

- SUSARs that have arisen in the clinical trial that was assessed by the EC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the EC.

The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the EC, CA and the Dutch Medicines Evaluation Board, a separate notification is not necessary. To prevent a double notification, it must be indicated in ToetsingOnline if the SUSAR is reported in the EMA EudraVigilance database, this will prevent the notification of the CA and the Dutch Medicines Evaluation Board through the web portal ToetsingOnline.

The investigator will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.1.8 Follow-up of adverse events

All AEs will be followed until they have abated, returned to baseline status or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

8.2 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the investigator will inform the subjects and the EC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the EC, except insofar as suspension would jeopardize the subjects' health. The investigator will ensure that all subjects are kept informed.

8.3 Annual safety report or development safety update report

In addition to the expedited reporting of SUSARs, the investigator will submit, once a year throughout the clinical trial, a safety report to the EC, CA and CAs of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9 STATISTICAL METHODOLOGY AND ANALYSES

9.1 Statistical analysis plan

All safety and statistical programming is conducted with SAS 9.4 for Windows or newer (SAS Institute Inc., Cary, NC, USA).

9.2 Protocol violations/deviations

Protocol deviations will be identified based on conditions related to the categories below:

- Protocol entry criteria
- Forbidden concomitant medications
- Missing evaluations for relevant endpoints
- Other protocol deviations occurring during study conduct.

Major protocol deviations will be identified before the study closure, and listed where appropriate.

9.3 Power calculation

Due to the nature of this exploratory trial, no formal power analysis has been conducted.

9.4 Missing, unused and spurious data

All missing or incomplete safety and pharmacodynamics data, including dates and times, are treated as such. Missing test results or assessments will not be imputed. Missing pharmacodynamics data, indicated as 'M' in the data listing, will be estimated within the statistical mixed model using SAS PROC MIXED.

For graphical and summary purposes pharmacodynamic and safety values below the limit of quantification will be set to half ($\frac{1}{2}$) of the limit of quantification. For analysis no undetermined values will be replaced.

The handling of missing, unused and spurious data will be documented in the study report.

9.5 Analysis sets

Data of all subjects participating in the study will be included in the analyses if the data can meaningfully contribute to the objectives of the study.

9.5.1 Pharmacodynamic analysis set

The analysis population for pharmacodynamics is defined as all subjects who were validated (randomized), underwent at least 1 skin punch biopsy, and have at least one post-baseline assessment of the parameter being analyzed.

9.5.2 Safety set

The safety population will be defined as all subjects who were validated (randomized) and underwent at least 1 skin punch biopsy.

9.6 Subject disposition

Subject disposition will be listed by subject.

The following subject data will be summarized:

- number and percentage of subjects screened,
- number and percentage of subjects enrolled,
- number and percentage of subjects completed,
- number and percentage of subjects included in safety population and
- number and percentage of subjects included in the pharmacodynamic analysis population.

A subject is defined to have completed the trial when he or she has all biopsies taken.

9.7 Baseline parameters and concomitant medications

9.7.1 Demographics and baseline variables

Continuous demographic variables (e.g., age, height, weight, BMI) will be summarized by descriptive statistics (n, mean, SD, median, Min, Max).

Qualitative demographic characteristics (sex, race/ethnicity) will be summarized by counts and percentages.

9.7.2 Medical history

Medical history will only be listed.

9.7.3 Concomitant Medications

All concomitant medications will be displayed in a listing.

9.7.4 Treatment compliance/exposure

Not applicable.

9.8 Safety and tolerability endpoints

The safety set is used to perform all safety analyses. Baseline is defined as the last value prior to dosing. Change from baseline will be calculated for all continuous safety parameters.

9.8.1 Adverse events

The AE coding dictionary for this study will be Medical Dictionary for Regulatory Activities (MedDRA). It will be used to summarize AEs by primary system organ class (SOC) and preferred term (PT).

All adverse events will be displayed in listings.

A treatment-emergent adverse event (TEAE) is defined as an adverse event observed after the first skin punch biopsy is taken. If a subject experiences an event both prior to and after the first skin punch biopsy, the event will be considered a TEAE (of the treatment) only if it has worsened in severity (i.e., it is reported with a new start date) after the first skin punch biopsy, and prior to the start of another treatment, if any. All TEAEs collected during the investigational period will be summarized.

The number of subjects with treatment emergent AEs will be summarized by:

1. treatment, MedDRA SOC and PT;
2. treatment, MedDRA SOC, PT and severity;
3. treatment, MedDRA SOC, PT and drug relatedness.

9.8.2 Vital signs

At each time point, absolute values and change from baseline of supine BP and HR will be summarized with n, mean, SD, SEM, median, Min, and Max values.

9.8.3 Clinical laboratory tests

At each time point, absolute values and change from baseline of clinical laboratory variables will be summarized with n, mean, SD, SEM, median, Min, and Max values. The number of available observations and out-of-range values (absolute and in percentage) will be presented. All laboratory data (including re-check values if present) will be listed chronologically.

The categorical data of the urinalysis will be summarized by treatment and time in frequency tables by variable.

9.9 Endpoints

The final analysis will be preceded by a data review which consists of individual graphs per visit by time of all pharmacodynamic measurements by time. The graphs will be used to detect outliers and measurements unsuitable for analysis.

The pharmacodynamic parameters will be listed by treatment, subject, visit and time. Individual graphs by time will be generated.

Given the exploratory character of the study, pharmacodynamic endpoints will be primarily analyzed using descriptive statistics. All pharmacodynamic endpoints will be summarized (mean and standard deviation of the mean, median, minimum and maximum values) by treatment and time, and will also be presented graphically as mean over time, with standard deviation as error bars. Both Nominal results, and log-transformed results and change from baseline results will be utilized in all data summaries. All categorical pharmacodynamic endpoints will be summarized by frequencies.

Parameters will initially be analyzed without transformation, but if the data suggest otherwise, log-transformation may be applied. Log-transformed parameters will be back-transformed after analysis where the results may be interpreted as percentage change.

9.9.1 Inferential methods

The study is exploratory and no formal null hypothesis is set. No adjustments for multiple comparisons will be applied.

9.10 Exploratory analyses and deviations

Exploratory data-driven analyses can be performed with the caveat that any statistical inference will not have any confirmatory value.

Deviations from the original statistical plan will be documented in the clinical study report.

9.11 Interim analyses

No interim analysis is planned.

10 GOOD CLINICAL PRACTICE, ETHICS AND ADMINISTRATIVE PROCEDURES

10.1 Good clinical practice

10.1.1 Ethics and good clinical practice

The investigator will ensure that this study is conducted in full compliance with the protocol, the principles of the Declaration of Helsinki (www.wma.net), ICH GCP guidelines (<http://www.ich.org/products/guidelines.html>), and with the laws and regulations of the country in which the clinical research is conducted.

10.1.2 Ethics committee / institutional review board

The investigator will submit this protocol and any related documents to an Ethics Committee (EC) and the Competent Authority (CA). Approval from the EC and the statement of no objection from the CA must be obtained before starting the study, and should be documented in a dated letter/email to the investigator, clearly identifying the trial, the documents reviewed and the date of approval. A list of EC members must be provided, including the functions of these members. If study staff were present, it must be clear that none of these persons voted.

Modifications made to the protocol after receipt of the EC approval must also be submitted as amendments by the investigator to the EC in accordance with local procedures and regulations.

10.1.3 Informed consent

It is the responsibility of the investigator to obtain written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives and potential hazards of the study. The investigator must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time for any reason.

The Informed Consent and Subject Information will be provided in Dutch.

10.1.4 Insurance

The investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The investigator also has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23rd June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

- € 650,000.- (i.e., six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
- € 5,000,000.- (i.e., five million Euro) for death or injury for all subjects who participate in the Research;
- € 7,500,000.- (i.e., seven million and five hundred thousand Euro) for the total damage incurred by the organization for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.2 Study funding

CHDR is the sponsor of the study and Cutanea Life Sciences is funding the study. All financial details are provided in the separate contract(s) between the CHDR and Cutanea Life Sciences.

10.3 Data handling and record keeping

10.3.1 Data collection

Data will be recorded mainly on electronic data collection forms. When necessary, paper data collection forms can be used and data will then be entered after quality control in a Promasys database for subsequent tabulation and statistical analysis. The data will be handled confidentially and if possible anonymously. A Subject Screening and Enrolment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.

10.3.2 Database management and quality control

All data from paper source will be entered into the Promasys database twice, by two different individuals. A quality control check will be done by CHDR staff on all data entered in the Promasys database, using data entry progress checks and database listings (blind data review). Errors with obvious corrections will be corrected before database lock. Results of computer tests and electronically captured questionnaires will be sent electronically to CHDR and loaded into the database. After the database has been declared complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement between the investigator and the statistician.

10.4 Access to source data and documents

All study data will be handled confidentially. The investigator will retain the originals of all source documents generated at CHDR for a period of 2 years after the report of the study has been finalized, after which all study-related documents will be archived (at a minimum) on micro-film which will be kept according to GCP regulations. After 2 years the sponsor will be notified that the source documents can be retained with the sponsor or destroyed.

The investigator will permit trial-related EC review and regulatory inspections, providing direct access to source data and documents.

10.5 Quality control and quality assurance

This study will be conducted according to applicable Standard Operating Procedures (SOPs). Quality assurance will be performed under the responsibility of CHDR's Quality Assurance manager.

10.5.1 Monitoring

Data monitoring will be performed by CHDR procedures including source data verification, trial master file verification, verification of delegation of authorities and appropriate training of personnel. According to CHDR procedures an initiation visit (pre-study meeting) will be performed before the first subject is included. Monitoring visits and contacts will occur at regular intervals thereafter, according to a frequency defined in the study-specific monitoring plan. A close-out visit will be performed after study closure.

10.6 Protocol amendments

Any change to a protocol has to be considered as an amendment.

10.6.1 Substantial amendment

Significant changes that affect subject safety and/or the scientific value of a trial require a substantial amendment. Examples of significant changes are given in EU guidelines on the request to the competent authorities for authorization of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1, 2010/C 82/01). The need for submitting a substantial amendment is the responsibility of the sponsor. Substantial amendments are to be approved by the appropriate EC and the CA will need to provide a 'no grounds for non-acceptance' notification prior to the implementation of the substantial amendment.

10.6.2 Non-substantial amendment

Non substantial amendments do not affect subject safety or the scientific integrity of the trial. Non-substantial amendments will be approved (signed) by the investigator(s) and will be recorded and filed by the investigator/sponsor. Non-substantial amendments will be submitted to the EC for information only. The CA will only be notified by changes in Eudract form and ABR form (if applicable) at toetsingonline. The implementation of a non-substantial amendment can be done immediately.

The EU guideline CT-1 2010/C 82/01 stipulates the importance of preventing over-reporting. Therefore the following changes are by definition non-substantial in this study:

- change in amount and timing of the samples (maximum of 2 samples without a > 50 ml increase in the amount of blood taken and not exceed 500 ml of blood in total)
- changes in assay-type and / or institution where an assay will be performed, provided that validated assays will be used;
- editorial changes to documents in the submission dossier including the volunteer information sheets and the protocol. An editorial change is defined as a modification in the documents of typographical errors and other modifications that in no way alter the meaning or content of the document
- determination of additional parameters in already collected materials, which are in agreement with the study objectives and do not provide prognostic or genetic information;
- other statistical analyses than described in the protocol.
- A change in clinical staff, including the principal investigator, when this concerns regular staff members of CHDR who comply with internal regulations for training and authorization.
- A change in dosing schedule in an ascending dose trial, provided the expected exposure of the subjects does not exceed the preset values indicated in this protocol.

10.6.3 Urgent amendment

An urgent amendment might become necessary to preserve the safety of the subjects included in the study. The requirements for approval should in no way prevent any immediate action being taken by the investigators in the best interests of the subjects. Therefore, if deemed necessary, an investigator can implement an immediate change to the protocol for safety reasons. This means that, exceptionally, the implementation of urgent amendments will occur before submission to and approval by the EC(s) and CA.

10.7 End of study report

The sponsor will notify the EC and the CA of the end of the study within a period of 90 days. The end of the study is defined as the last subject's last visit. In case the study is ended prematurely, the

investigator will notify the EC and the CA within 15 days, including the reasons for the premature termination.

CHDR will notify the EC immediately of a temporary halt of the study, including the reason of such an action.

Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the EC and the CA. The principal investigator will be the signatories for the study report.

10.8 Public disclosure and publication policy

In accordance with standard editorial and ethical practice, the results of the study will be published. The authorship guidelines of the Vancouver Protocol³ will be followed regarding co-authorship.

³ <http://www.icmje.org/>

11 STRUCTURED RISK ANALYSIS

No drug or treatment with active ingredients is to be used in this study.

12 REFERENCES

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- ILLIGENS, B. M. & GIBBONS, C. H. 2013. A human model of small fiber neuropathy to study wound healing. *PLoS One*, 8, e54760.
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- UD-DIN, S., PERRY, D., GIDDINGS, P., COLTHURST, J., ZAMAN, K., COTTON, S., WHITESIDE, S., MORRIS, J. & BAYAT, A. 2012. Electrical stimulation increases blood flow and haemoglobin levels in acute cutaneous wounds without affecting wound closure time: evidenced by non-invasive assessment of temporal biopsy wounds in human volunteers. *Exp Dermatol*, 21, 758-64.

APPENDIX A

CHDR1736 POSAS Observer Scale (English)

POSAS Observer scale

The Patient and Observer Scar Assessment Scale v2.0 / EN

Date of examination:

Name of patient:

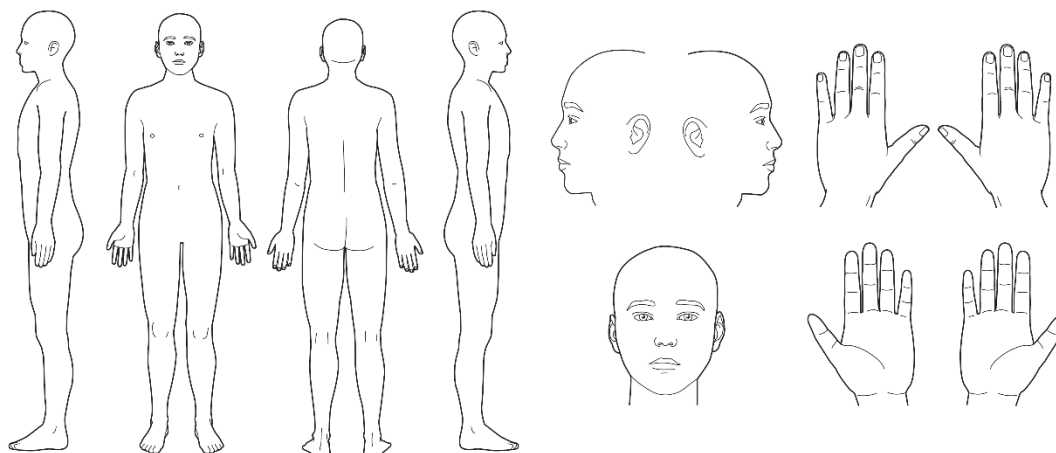
Observer:

Date of birth:

Location:

Identification number:

Research / study:



	1 = normal skin worst scar imaginable = 10										
PARAMETER	1	2	3	4	5	6	7	8	9	10	CATEGORY
VASCULARITY	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	PALE PINK RED PURPLE MIX
PIGMENTATION	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	HYPO HYPER MIX
THICKNESS	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	THICKER THINNER
RELIEF	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	MORE LESS MIX
PLIABILITY	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	SUPPLE STIFF MIX
SURFACE AREA	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	EXPANSION CONTRACTION MIX
OVERALL OPINION	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	

Explanation

The observer scale of the POSAS consists of six items (vascularity, pigmentation, thickness, relief, pliability and surface area). All items are scored on a scale ranging from 1 ('like normal skin') to 10 ('worst scar imaginable'). The sum of the six items results in a total score of the POSAS observer scale. Categories boxes are added for each item. Furthermore, an overall opinion is scored on a scale ranging from 1 to 10. All parameters should preferably be compared to normal skin on a comparable anatomic location.

Explanatory notes on the items:

- **VASCULARITY** Presence of vessels in scar tissue assessed by the amount of redness, tested by the amount of blood return after blanching with a piece of Plexiglas
- **PIGMENTATION** Brownish coloration of the scar by pigment (melanin); apply Plexiglas to the skin with moderate pressure to eliminate the effect of vascularity
- **THICKNESS** Average distance between the subcuticular-dermal border and the epidermal surface of the scar
- **RELIEF** The extent to which surface irregularities are present (preferably compared with adjacent normal skin)
- **PLIABILITY** Suppleness of the scar tested by wrinkling the scar between the thumb and index finger
- **SURFACE AREA** Surface area of the scar in relation to the original wound area

APPENDIX B

CHDR1736 POSAS Observer Scale (Dutch)

POSAS Observer scale

The Patient and Observer Scar Assessment Scale v2.0 / NL

Datum onderzoek:

Naam:

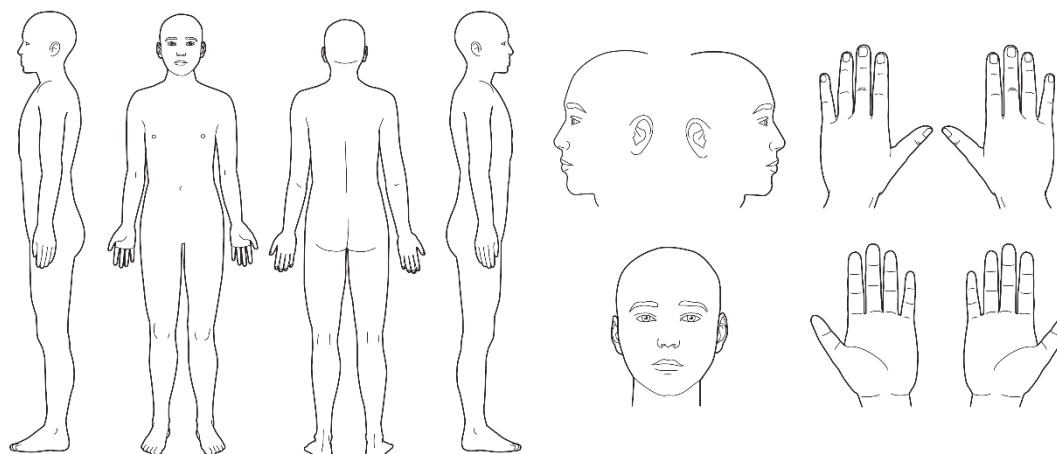
Beoordelaar:

Geboortedatum:

Locatie:

Patientennummer:

Studie:



	1 = normale huid ergst denkbare litteken = 10										
PARAMETER	1	2	3	4	5	6	7	8	9	10	CATEGORIE
VASCULARISATIE	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	BLEEK ROZE ROOD PAARS MIX
PIGMENTATIE	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	HYPO HYPER MIX
DIKTE	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	DIKKER DUNNER
RELIËF	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	VLAKKER HOBBELIGER MIX
PLOOIBAARHEID	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	STUGGER SOEPELER MIX
OPPERVLAKTE	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	EXPANSIE CONTRACTIE MIX
ALGEGELE INDRUK	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	

Toelichting

De POSAS bestaat uit de zes items (vascularisatie, pigmentatie, dikte, reliëf, plooibaarheid en oppervlakte) waarvan het litteken gescoord wordt op een schaal van 1 (als normale huid) tot 10 (als de ergste denkbare afwijking). De score van deze zes items resulteert in de 'POSAS totaal score'. Achter elk van de zes items staan categorieën vermeld die ingevuld kunnen worden. Daarnaast wordt de algemene indruk gescoord op een schaal van 1 tot 10. Voor alle parameters geldt dat indien mogelijk een vergelijking dient plaats te vinden met normale huid op een overeenkomstige anatomische locatie.

Toelichting op de items:

- **VASCULARISATIE** De vascularisatie wordt beoordeeld aan de hand van wegdrukbaar roodheid. Dit wordt getest door met Plexiglas voldoende druk aan te brengen om de vaten in het litteken dicht te drukken en vervolgens het Plexiglas los te laten.
- **PIGMENTATIE** De mate van bruine verkleuring van het litteken door de aan- of afwezigheid van melanine. Het plexiglas wordt tijdelijk op het litteken aangebracht met voldoende druk om de vaten in het litteken dicht te drukken waardoor de pigmentatie beter beoordeeld kan worden.

- **DIKTE** De gemiddelde afstand tussen de overgang subcutis – dermis en de epidermale oppervlakte van het litteken.
- **RELIËF** De mate waarin oppervlakte onregelmatigheden van het litteken aanwezig zijn (Indien mogelijk wordt dit vergeleken met normale huid op een overeenkomstige anatomische locatie).
- **PLOOIBAARHEID** De soepelheid van het litteken. Dit wordt getest door de littekenhuid tussen duim en wijsvinger te plooiën.
- **OPPERVLAKTE** De oppervlakte van het litteken in relatie tot het oorspronkelijke wondgebied.

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